

Dear iGEM team,

You just received an encrypted message in our CryptoGERM system. In order to read out our secret message follow the instructions in this letter.

Content:

the CryptoGERM	aka <i>Bacillus subtilis</i> spores including the encrypted message in form of a DNA sequence integrated in the genome. Find tube labeled CryptoGERM.
biological key	Primers that will amplify the message, therefore the key to the right sequence. Find tube labeled forward key and reverse key.
digital key	needed to decrypt the message, once the right sequence was found. For this test we will just tell you the key: Autoclave after reading!
decrypter	our decoding program, follow this link: http://2016.igem.org/Team:Groningen/FullCoding

Instructions:

1. Germinate the cryptoGERM. Therefore add 100 μ l LB in the tube labeled cryptoGERM and vortex. Take 50 μ l and plate the cryptoGERM on an LB agar with 150 μ g/ml spectinomycin, if you do not have this antibiotic LB agar without should also be fine. Incubate the plate overnight at 37°C and fingers crossed for colonies.



Figure 1: The CryptoGERM

2. Amplify the message sequence with the biological key from the CryptoGErM:

PCR Master mix:

Component	Volume
Buffer	
dNTPs	
water	
Forward key (10 μ M)	1 μ l
Reverse key (10 μ M)	1 μ l
cryptoGErM colony	Dilute colony in 50 μ l water, vortex and use 2 μ l of that
MiliQ	Fill up to 20 μ l
Polymerase (high fidelity)	

Make sure you use a high fidelity polymerase to prevent mutations.

PCR cycling conditions:

95°C 2:00 min
95°C 30s (30X)
63°C 30s (30X)
72°C * (30X)
72°C 2:00 min
10°C on hold

* you are looking for a 916 bp large PCR product. Make sure your polymerase has enough elongation time. For us it was 1 min.

3. Check with gel electrophoresis if you get a band at 916 bp. Load 5 μ l.

4. Use the leftover 15 μ l PCR product. Prepare two tubes for sequencing

1. Forward key 5 μ l + 5 μ l PCR product
2. Reverse key 5 μ l + 5 μ l clean PCR product.

Follow the instructions of your Send for sequencing!

5. Receive the sequence and enter it to our decrypter. Now you need the digital key to decrypt the message, just enter it in the key field.

6. Send us a selfie with you and the decrypted message!

Happy decrypting,

the Groningen iGEM team 2016

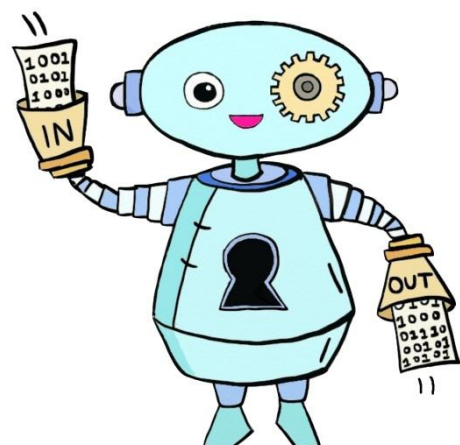


Figure 2: The decrypter